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Starch-synthesizing Enzymes in the Endosperm and Pollen of Maize[1, 2, 3]

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ABSTRACT

Two mutations, *amylose-extender* and *waxy*, which affect the proportion of amylose and amylopectin of starch synthesized in the endosperm of maize (*Zea mays* L.) seeds, are also expressed in the pollen. However, most mutations that affect starch synthesis in the maize endosperm are not expressed in the pollen. In an attempt to understand the nonconcordance between the endosperm and pollen, extracts of mature pollen grains were assayed for a number of the enzymes possibly implicated in starch synthesis in the endosperm. Sucrose synthetase (sucrose-UDP glucosyl transferase, EC 2.4.1.13) activity was not detectable in either mature or immature pollen grains of nonmutant maize, but both bound and soluble invertase (EC 3.2.1.26) exhibited much greater specific activity (per milligram protein) in pollen extracts than in 22-day-old endosperm extracts. Phosphorylase (EC 2.4.1.1) activity was also higher in pollen than in endosperm extracts. ADP-Glucose pyrophosphorylase (EC 2.7.7.27) activity was much lower in pollen than endosperm extracts, but mutations that drastically reduced ADP-glucose pyrophosphorylase activity in the endosperm (*brittle-2* and *shrunken-2*) did not markedly affect enzymic activity in the pollen. Specific activities of other enzymes implicated in starch synthesis were similar in endosperm and pollen extracts.

The *waxy* mutation affects the type of starch produced by mutant endosperm tissue, pollen grains, and megaspores, but does not affect the starch produced by sporophytic tissue (4, 5, 9). By contrast, many mutations (*e.g. brittle, brittle-2, shrunken, shrunken-2, shrunken-4, sugary*) that drastically reduce starch synthesis in the endosperm apparently do not do so in pollen grains. This is inferred from the normal size and staining properties of mutant pollen and from good agreement with the expected 3 normal to 1 mutant ratio in the progeny of heterozygous plants. Among other factors, this ratio depends upon the mutant pollen grains being fully competitive with nonmutant pollen grains in effecting fertilization. Two mutants, *shrunken-2* and *sugary*, are known to produce normal levels of starch in the pollen grains (26).

One possible explanation for the tissue specificity of the mutant effects is that a necessary enzyme is coded by a different gene(s) in each tissue with each gene(s) being expressed in only one tissue. The genes are thus coding for nonallelic isozymes. Mutant genes that affect the starch in the developing maize endosperm without altering the sporophytic starch include *waxy* (1), *shrunken* (8), and *shrunken-2* (27). For each of these mutants, the tissue-specific expression has been attributed to a given enzyme being encoded by different genes in the endosperm and the sporophyte.

A second possible explanation is that starch is synthesized in the pollen via a pathway that differs in one or more steps from the pathway active in the endosperm. The effect of *waxy* mutants on starch synthesis in both tissues indicates that there must be some common steps and that at least one enzyme active in both tissues is coded by only one gene.

In this investigation, we have examined in extracts of nonmutant and mutant pollen grains the activities of a group of enzymes implicated in endosperm starch synthesis. This was done in the hope of ascertaining if nonallelic isoenzymes, differences in the pathways of starch synthesis, or both, might account for the observed differences in the mutants.

MATERIALS AND METHODS

Source of Pollen and Endosperm. Maize (*Zea mays* L.) seeds which were homozygous for one of the following endosperm starch mutations that had been recovered from an outcross to the hybrid, W64A × 182E, were grown in a field in Madison, Wisconsin: *sh2*,[5] *bt2, sh4, ae,* and *wx*.

Mature pollen was collected from the normal (nonmutant) hybrid W64A × 182E and from the mutants. Pollen was passed through a 30-mesh screen to remove anthers, immediately frozen on dry ice, and stored at −20 C.

Immature tassels of the normal hybrid were gathered 1 or 2 weeks prior to anthesis (pollen shedding) and frozen on dry ice. This material was used to test for sucrose synthetase activity as described later.

Controlled self-pollinations of the normal hybrid and the mutants were made. Ears were harvested and quickly frozen 22 days after pollination.

Soluble Enzyme Extraction Procedures: Mature Pollen. Pollen samples (approximately 2.5 g fresh weight) were weighed and then homogenized in chilled 10 mM Tris-HCl (pH 7.5) (containing 1 mM DTT and 0.1 mM EDTA) in the ratio of 1 g pollen/5 ml extraction buffer. All extractions, centrifugations, and dialyses were done at 4 C. The homogenization was done using a Ten-Broeck tissue grinder.

The homogenate was strained through two layers of cheesecloth and centrifuged for 25 min at 39,100*g*. The resulting pellet was saved and after further treatment was used to assay for particulate enzymes, including bound invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26), bound P-glucomutase (α-D-glucose-1,6-diP: α-D-glucose-1-P phosphotransferase, EC 2.7.5.1), and the starch granule-bound nucleoside diP glucose-starch glucosyltrans-

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[5] Abbreviations: *ae: amylose extender; bt2: brittle-2; sh: shrunken-1; sh2: shrunken-2; sh4: shrunken-4; wx: waxy.* Endosperms of genotype *sh2/sh2/sh2* are designated as *sh2* endosperms while endosperms not carrying a mutant gene are designated as normal or +.

ferase (nucleoside diP-glucose: α-1,4-glucose α-4-glucosyltransferase). The supernatant fraction was dialyzed overnight against 2 liters of extraction buffer and used to assay for the following enzymes: soluble invertase, sucrose synthetase (UDP-glucose: D-fructose-2 glucosyltransferase, EC 2.4.1.13), soluble P-glucomutase, glucose-P isomerase (D-glucose-6-P ketoisomerase, EC 5.3.1.9), hexokinase (ATP: D-hexose-6-P-transferase, EC 2.7.1.1), nucleoside diP kinase (ATP: nucleoside diP P-transferase, EC 2.7.4.6), soluble ADP-glucose-starch glucosyltransferase (ADP-glucose: α-1,4-glucose α-4-glucosyltransferase), and sucrose-6-P synthetase (UDP-glucose: D-fructose-6-P-2-glucosyltransferase, EC 2.4.1.14).

ADP-Glucose pyrophosphorylase (ATP: α-glucose-1-P adenyltransferase, EC 2.7.7.27) and UDP-glucose pyrophosphorylase (UTP: α-glucose-1-P uridyltransferase, EC 2.7.7.9) were prepared using the extraction and dialysis buffers of Dickinson and Preiss (10). Enzyme preparations used in assaying α-1,4-glucose: Pi glucosyltransferase, EC 2.4.1.1) were obtained as described by Burr and Nelson (6, 7).

Activities for all enzymes are measured for two different preparations with at least three replicates per assay. Data are presented as the mean activity for both preparations.

Immature Pollen Sucrose Extraction. Anthers were separated from glumes of immature maize tassels at 4 C. The immature anthers were extracted utilizing the same conditions given above for mature pollen sucrose synthetase.

Immature Endosperm Enzyme Extraction. Following pericarp and embryo removal from 22-day-old kernels, the endosperms were weighed and homogenized in a VirTis 45 blender in a ratio of 1 g fresh weight to 1 ml extraction buffer. Except for this modification, the procedures followed were identical to those described for the corresponding pollen enzymes.

Mature Endosperm Sucrose Synthetase Extraction. Embryos but not pericarp were removed from mature, unsoaked seed, and the separated endosperms were weighed, counted, and then ground into a fine meal in a Wiley mill with a 20-mesh sieve and finally by a cyclone mill. About 2.5 g of the meal were suspended in extraction buffer in the ratio of 1 g endosperm meal to 3 ml buffer and stirred at low speed for 6 hr at 4 C. This suspension was centrifuged at 39,100*g* for 20 min. The supernatant was dialyzed 15 hr against 2 liters of extraction buffer and used as a source of enzyme.

Particulate Enzymes. Three particulate enzymes were measured in pollen and endosperm tissues. They were bound invertase, bound P-glucomutase, and the starch granule-bound nucleoside diP glucose-starch glucosyltransferase (bound starch synthetase).

Bound invertase and bound P-glucomutase were obtained from the initial 39,100*g* pellet by resuspending it in extraction buffer. This suspension was centrifuged again at 39,100*g* for 25 min and the supernatant fraction decanted. This process was repeated five times. As the final step, the pellet was suspended in acetone, stirred, and centrifuged. The supernatant fraction was decanted and the pellet air-dried.

The initial 39,100*g* pellet was also the source of the starch granule-bound starch synthetase. For endosperm, the overlaying yellow layer (chromatin and carotenoid pigments) was scraped off the pellet after each of the six centrifugations. For the final step, the pellet was suspended in acetone and treated as described in the previous paragraph.

For pollen, it was necessary to start with 20 to 25 g (fresh weight) pollen. The homogenate was centrifuged at 27,300*g* for 30 min. As much as possible of the yellowish material in the pellet was scraped off. The pollen starch was visible as a white band which ran parallel to the upper edge of the pellet. Five repetitions of the resuspension and centrifugation methods previously described were followed by a siphoning step which removed the remaining cell debris from the pollen starch granule preparation. Acetone resuspension and subsequent steps were performed as outlined in the previous paragraphs. The final pellet was free of the yellowish substance.

All starch preparations were stored at 4 C in a desiccator.

Enzyme Assay Methods. For all soluble enzymes, the dialyzed crude 39,100*g* supernatant fraction was the source of enzyme, except for soluble ADP-glucose-starch glucosyltransferase, in which a 0 to 40% ammonium sulfate fraction was taken as described by Ozbun *et al.* (25).

Soluble invertase, soluble P-glucomutase, and glucose-P isomerase were assayed as described by Tsai *et al.* (32). Soluble sucrose synthetase activity was determined in the direction of sucrose synthesis using the procedure of Tsai *et al.* (32).

Sucrose synthetase was assayed in the direction of sucrose breakdown in a reaction mixture which consisted of 60 μmol MES (pH 6.5), 1.5 μmol UDP, 240 μmol sucrose, and pollen preparation in a final volume of 550 μl. The control was a reaction mixture from which UDP was omitted.

Sucrose synthetase activity was also assayed using the extraction and dialysis buffers of Slack (28). The extraction buffer contained 10 mM sodium diethyldithiocarbamate and 0.2 M K-phosphate (pH 6.8). Sucrose-6-P synthetase activity was measured in the direction of sucrose-6-P synthesis as in reference 29.

Nucleoside diP kinase and hexokinase activities were determined as in (12). ADP-Glucose pyrophosphorylase activity was measured following the procedure of Dickinson and Preiss (10) as modified by Hannah and Nelson (13). UDP-Glucose pyrophosphorylase was assayed by the above method with the difference that UTP replaced ATP in the reaction mixture.

The assay method of Tsai and Nelson (31) was used for phosphorylase except that 2 mg phytoglycogen replaced 5 mg amylopectin as the polysaccharide primer.

Primed soluble ADP-glucose-starch glucosyltransferase was measured according to the procedure of Ozbun *et al.* (25) except that 2 mg of phytoglycogen served as the acceptor instead of amylopectin.

Starch granule-bound nucleoside diP glucose-starch glucosyltransferases were measured by the above method, but phytoglycogen was omitted. Three mg of pollen starch granules or 3 mg of endosperm starch granules were incubated 15 to 30 min at 37 C.

Amylose and Starch Determinations. The amylose content of pollen and endosperm starch was estimated using the procedure of Ulmann and Augustat (33) starting with 100-mg samples of pollen or endosperm starch granules.

Starch content of seeds was determined by the McGuire and Erlander (20) method after pericarp and embryos were removed from mature kernels. The endosperm meal was washed with *n*-hexane in a Soxhlet apparatus for 36 hr. Pollen starch content was ascertained by a modification of this procedure in which lyophilized pollen was homogenized in chilled distilled H_2O in a Ten-Broeck tissue grinder and immediately frozen in an acetone-dry ice bath and lyophilized. This lyophilized pollen sample was then treated as the endosperm samples.

Protein Determinations. Soluble protein was determined by the method of Lowry *et al.* (19). The micro-Kjeldahl procedure outlined in AOAC (2) was used to determine the nitrogen content of lyophilized pollen and endosperm, and the cellular debris used to assay for bound invertase and P-glucomutase.

RESULTS AND DISCUSSION

Pollen Composition Data. Normal, *sh2*, and *sh4* pollen were very similar in starch and soluble and total protein content (Table I). It is apparent that the mutants *sh2* and *sh4* do not significantly lower the starch content of the pollen as they do in endosperms (17, 24). This was previously noted for *sh2* pollen (26).

Comparable levels of soluble protein were observed in *sh4* and normal pollen. The soluble protein content of *sh4* endosperms has been reported (24) to be reduced to about 30% of the level of

normal in a W64A background. It is clear that neither the reduced starch content nor the soluble protein deficiency associated with *sh4* in the endosperm is expressed in the pollen.

A mutation which does influence the starch composition of the pollen is *amylose-extender* (*ae*). About 35% of the starch of *ae* pollen is amylose; starch isolated from *ae* endosperms 22 days after pollination was found to contain about 35 to 40% amylose (Table II). Pollen or endosperm starch derived from normal plants contained about 25% amylose. These findings support the assertion by Banks *et al.* (3) that there is a positive correlation in the amylose content of starch from the endosperm and pollen grains of *ae* maize.

Survey of Enzymes in Pollen. A survey in mature pollen for several enzymes that appear to participate in endosperm starch synthesis was conducted. With the exception of sucrose-UDP-glucosyltransferase, all of the enzymes associated with endosperm starch synthesis were active in pollen.

Invertase. In addition to soluble invertase, an insoluble form of invertase has been described in maize coleoptiles (16). As can be seen in Table III, both soluble and bound forms of acid invertase were found in pollen and endosperms. Pollen exhibited much higher acid invertase activity per mg protein that was found in the endosperm.

Sucrose Synthetase. While high levels of both soluble and bound invertase were observed, no detectable activity for sucrose synthetase was found in mature pollen (Tables III and IV). Both mature and developing endosperms (22 days after pollination), on the other hand, have readily detectable sucrose synthetase activities. Developing endosperms had about five times the sucrose synthetase levels found in mature endosperms when activities were expressed on a mg protein basis. Chourey and Nelson (8) have shown that developing *sh* endosperms, harvested 22 days after pollination, had less than 10% as much sucrose synthetase activity as did normal endosperms. Mature W22 *sh* endosperms also have reduced sucrose synthetase activity as compared to normal W22 (+) endosperms (Table IV). In mature W22 *sh* endosperms, the sucrose synthetase activity was about 1% of that observed in normal W22 (+) endosperms. There was no detectable activity in mature pollen for sucrose synthetase or in immature pollen from tassels 1 or 2 weeks prior to anthesis. At these same stages of pollen development, ADP-glucose pyrophosphorylase and invertase activities were detectable (data not given).

The data shown in Table IV are sucrose synthetase activities measured in the direction of sucrose synthesis. It is possible that there was some soluble sucrose synthetase activity present in pollen, but the sucrose produced was hydrolyzed by invertase. To exclude this possibility, sucrose synthetase was assayed in the direction of sucrose breakdown. No detectable sucrose synthetase activity was present.

Another possibility is that sucrose synthetase in maize pollen is an enzyme tightly bound to a particulate fraction. When a pollen particulate preparation was assayed for sucrose synthetase activity

Table I. Protein and starch content of non-mutant and mutant pollen grains

Values are means of 2 assays, 3 replicates per assay.

Genotype	Protein		Starch
	Soluble	Total	
	% of dry weight		
+ (normal)	6.06	21.3	24.3
sh2	6.12	22.3	24.7
sh4	5.51	22.6	22.3

Table II. Amylose percentages of starch from 22-day-old endosperm and mature pollen

Values are means of 2 assays, 3 replicates per assay.

Year	Source of starch	Amylose content of starch
		%
1972	+ pollen	26.0
1972	+ endosperm	24.2
1972	ae pollen	35.1
1972	ae endosperm	35.7
1974	ae endosperm	39.3

Table III. Soluble and bound invertase activities of normal pollen and endosperm measured at pH 4.8

Data presented in Tables III through VII are the means of 2 assays, with at least 3 replicates per assay.

Source of enzyme	Invertase activity
	μmol reducing sugar/min·mg protein
+ Pollen (soluble)	0.24
+ Pollen (bound)	0.76
+ Endosperm (soluble)	0.021
+ Endosperm (bound)	0.003

for incubation periods up to 60 min, no sucrose synthetase activity was found. Insoluble sucrose synthetases have not been reported in the literature, and sucrose synthetase was present as a soluble enzyme in the pollen of a number of species (*Lilium, Tulipa, Camellia,* and *Pinus*) (21).

It is not known whether sucrose synthetase was not formed in maize pollen or was inactivated rapidly. Sucrose synthetase in maize pollen would have to be completely inactivated in order to account for the total absence of measurable activity. Slack (28) reported that sucrose synthetase was inactivated by oxidation products of phenolic compounds released during homogenization of sugarcane stems. Diethyldithiocarbamate in the extraction buffer was reported to prevent the inactivation of sucrose synthetase. When the extraction and dialysis buffers of Slack (28) were used to obtain an enzyme preparation, no sucrose synthetase activity could be detected.

The possibility that sucrose-6-P synthetase (EC 2.4.1.14), an enzyme which catalyzes the reaction UDP-glucose + fructose-6-P → sucrose-P + UDP, may function in sucrose synthesis in maize pollen was also investigated. No detectable sucrose-P synthetase activity was present when maize pollen preparations were assayed for periods up to 60 min (data not given). Tsai (29) reported very low sucrose-P synthetase activities in maize endosperms.

Since sucrose synthetase activity is not observable either in mature pollen grains or immature pollen grains, its role in most starch-synthesizing tissues of making sucrose available for starch synthesis is probably taken by invertase for which high activity is observed. The high activities of both bound and soluble invertase may indicate their importance in pollen metabolism. Lendzian and Schaefer (18) postulated that the high level of bound invertase in *Haemanthus* pollen is concerned with the hydrolysis of stylar sucrose by germinating pollen. Hydrolysis of sucrose into hexoses may facilitate the rapid entry of sugars through the intine. In addition, the glucose and fructose produced by invertase would be channeled into several important metabolic steps besides starch synthesis such as glycolysis, the pentose-P pathway, and cell wall polysaccharide biosynthesis. These other pathways would be especially important during pollen germination and rapid pollen tube growth.

ADP-Glucose Pyrophosphorylase. The ADP-glucose pyrophosphorylase activities for pollen and endosperms homozygous for *sh2, bt2,* or their normal alleles are given in Table V. As mentioned earlier, ADP-glucose pyrophosphorylase activity is drastically reduced in *sh2* and *bt2* endosperms (11, 14, 30). The *sh2* endosperms had about 5%, and *bt2* endosperms about 6% of the ADP-glucose pyrophosphorylase activity present in normal endosperms (Table V). The ADP-glucose pyrophosphorylase activities of *sh2* and *bt2* pollen were somewhat lower than normal pollen, but the reduction was not nearly as pronounced as that noted in the endosperm. The specific activity in normal endosperms is far greater than that observed in normal pollen grains. It cannot be ascertained from these data whether *sh2* and the *bt2* mutations do condition some reduction in pollen ADP-glucose pyrophosphorylase activity or whether differences in background genotype could be responsible for the reductions noted. The somewhat lower enzyme activity in *sh2* is not reflected in lower pollen starch content (Table I).

Since the evidence is that these two loci are structural genes for the endosperm pyrophosphorylase (14) and since the enzyme is produced at nearly normal levels in the pollen of the mutants, we conclude tentatively that it is a product of loci other than *bt2* and *sh2.*

UDP-Glucose Pyrophosphorylase. The UDP-glucose pyrophosphorylase activity of normal endosperms and normal and *sh2* pollen is also shown in Table V. UDP-Glucose pyrophosphorylase

Table IV. Sucrose-UDP glucosyltransferase activities of mature normal (W64A x 182E) pollen and endosperms and 22-day developing normal (W64A x 182E) endosperms

Mature normal W22 endosperms and mature W22 sh endosperm preparations are also compared for sucrose synthetase activity.

Source of enzyme	Sucrose-UDP transferase activity
	μmol sucrose/min·mg protein
Mature pollen + (W64A x 182E)	<0.001
22-day endosperm + (W64A x 182E)	0.640
Mature endosperm + (W64A x 182E)	0.124
Mature endosperm + (W22)	0.231
Mature endosperm sh(W22)	0.002

Table V. ADP-glucose pyrophosphorylase and UDP-glucose pyrophosphorylase activity of normal (+), sh2, and bt2 endosperms and pollen

Preparations were incubated for 10 min at 37C.

Source of enzyme		ADP-glc pyrophosphorylase	UDP-glc pyrophosphorylase
		nmol glucose transferred/min·mg protein	
Pollen	+	5.05	430
Pollen	sh2	3.45	409
Pollen	bt2	2.36	
Endosperm	+	116.36	734
Endosperm	sh2	5.99	
Endosperm	bt2	7.37	

is considerably more active than ADP-glucose pyrophosphorylase in both pollen and endosperms. Similar UDP-glucose pyrophosphorylase activities were observed for normal and *sh2* pollen. Also, *sh2* and normal endosperms have equally high UDP-glucose pyrophosphorylase activity (30). The product of this pyrophosphorylase reaction, UDP-glucose, has been postulated to be a glucosyl donor in callose (15) and cell wall (12) formation so the enzyme could have an important function in pollen tube wall synthesis.

Starch-Glucosyltransferases. The *wx* endosperm is deficient in the bound glucose-starch glucosyltransferase (22). Nelson and Tsai (23) assayed normal and *wx* endosperms at 16 days after pollination for starch granule-bound nucleoside diP glucose-starch glucosyltransferase and noted that the mutant endosperms at this stage had only about 9% as much activity as nonwaxy. Activity for the starch granule-bound nucleoside diP glucose-starch glucosyltransferase from *wx* pollen was greatly reduced as it was in *wx* endosperms (Table VI). The *wx* endosperms at 22 days exhibited only 5% the level of bound starch glucosyltransferase present in normal endosperms, while in *wx* pollen the enzyme was about 8% as active as normal pollen. The activity of the soluble ADP-glucose-starch glucosyltransferase from *wx* pollen is equal to or greater than that of normal (Table VI). So the *wx* mutation appears to affect the bound starch glucosyltransferase in both the pollen and endosperm, but does not appear to alter drastically the activity of the soluble ADP-glucose-starch glucosyltransferase in these tissues. The bound nucleoside diP glucose-starch glucosyltransferase appears to play an important role in amylose synthesis in both the endosperm and pollen.

Other Enzymes. Nucleoside diP kinase activity was similar in both tissues (Table VII). Hexokinase, bound P-glucomutase, and glucose-P isomerase, were similar in both pollen and endosperms (Table VII). The soluble P-glucomutase from endosperms was twice as active per mg protein as that from mature pollen. Since pollen apparently does not have sucrose synthetase, hexokinase, the P-glucomutases, and glucose-P isomerase may be involved in

Table VI. The activity of starch granule-bound starch synthetase in non-mutant and wx pollen grains and developing endosperms and soluble starch synthetase activity in non-mutant and wx pollen grains

A concentration of 0.7 mM ADP-glucose was used in these assays.

	Source of enzyme	Starch granule-bound: nmol glucose incorp/30 min·mg starch granules	Soluble; nmol glucose incorp/30 min·mg protein
Pollen	+	14.20	53.3
Pollen	wx-B7	1.13	70.9
Endosperm	+	5.15	
Endosperm	wx-C[a]	0.28	
Endosperm	wx-B7	0.32	

[a] wx-C is the reference wx allele. B7 is a spontaneous wx mutant of independent origin.

Table VII. The activities of phosphorylase, nucleoside diphosphate kinase, and selected glycolytic enzymes from mature normal pollen and 22-day-old endosperms

Enzyme	Source of enzyme	Activity[1]
		μmol product formed/min·mg protein
Phosphorylase	+ pollen	51.6
	+ endosperm	17.3
Nucleoside diphosphate kinase	+ pollen	1.22
	+ endosperm	1.86
Hexokinase	+ pollen	0.023
	+ endosperm	0.025
Soluble phosphoglucomutase	+ pollen	0.64
	+ endosperm	1.26
Bound phosphoglucomutase	+ pollen	0.032
	+ endosperm	0.031
Glucose phosphate isomerase	+ pollen	1.34
	+ endosperm	1.19

[1] Activities are expressed in terms of μmol of the products of the enzyme-catalyzed reaction which are:

Phosphorylase: ^{14}C glucose incorporated into a phytoglycogen primer; nucleoside diphosphate kinase: ATP; hexokinase: glucose-6-phosphate; soluble and bound phosphoglucomutase: glucose-6-phosphate; and glucose phosphate isomerase: fructose-6-phosphate.

the production of substrate (glucose-1-P) for the pyrophosphorylase enzymes or phosphorylase. Phosphorylase activity from normal pollen was three times that of 22-day endosperms (per mg protein) (Table VII).

In comparisons of the specific activity of enzymes in extracts from nonmutant endosperms and nonmutant pollen grains, four obvious differences were observed. Extracts from pollen grains had no detectable sucrose synthetase activity, much lower ADP-glucose pyrophosphorylase activity, greatly increased invertase activity, and elevated phosphorylase levels.

The two mutants in which the effect on starch synthesis in the endosperm is extended to pollen grains are those in which the proportions of amylose and amylopectin are altered. Mutants which condition drastic disruptions in endosperm starch synthesis did not appear to affect pollen starch synthesis. For ADP-glucose pyrophosphorylase, the evidence is that the enzyme is coded in the endosperm and pollen by nonallelic genes. For this enzyme and probably for others whose absence would markedly reduce starch synthesis, different genes code for endosperm and pollen enzymes. These observations may be best explained by the assumption that, in the course of evolution, a set of duplicate starch-synthesizing genes has evolved that optimizes endosperm starch synthesis. It is possible that the form in which the starch occurs is insufficiently important to warrant selection for genes that are specialized for function in the endosperm or gametophyte alone.

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